AIF (YNR074C) is localized in yeast mitochondria but translocates to the nucleus during yeast cell death where it is suggested to damage/degrade DNA. Furthermore, overexpression of yeast AIF in yeast cells induces death that is inhibited by deletion of the metacaspase Yca1 or of yeast cyclophilin A (CypA). This is consistent with the reported role of cyclophilin A in AIF-mediated mammalian cell death, but is somewhat distinct from the proposed caspase-independent function of mammalian AIF. Other mysteries also remain, as yeast AIF shares greater sequence similarity with AMID, an AIF-like protein in mammals. Because sequence similarity between mammalian and yeast AIF is relatively low and limited to the oxidoreductase domain, there are other close contenders in yeast, such as YPL091W. Nevertheless, the yeast model will serve to investigate the yet unknown biochemical function of AIF that mediates cell death.

Fannjiang et al. (2004) identified another mitochondrial death pathway shared between yeast and mammals (Figure 1A). Mitochondrial fragmentation is often an early feature of programmed cell death in flies and mammals, and regulators of mitochondrial fission, such as Drp1 and Fis1, were shown to regulate cell death and cytochrome *c* release (Karbowski and Youle, 2003). We found that homologs of these mitochondrial fission factors also regulate yeast cell death (Fannjiang et al., 2004). However, mitochondrial fission alone is not sufficient to kill cells, but subsequent events mark the commitment point to death. Despite these analogies, several factors mentioned above, including AIF, caspases, Bcl-2, Drp1, and Fis1, can have opposite effects on cell survival under different circumstances. Yeast will surely serve as a powerful tool for dissecting both the basic mechanisms and the complexities of programmed cell death.

Why Would Yeast Want to Die?

If programmed cell death is defined as a molecular pathway that evolved for the purpose of cell suicide, then what is the evidence that yeast have a purpose in dying? While several groups have attempted to explain the benefits of programmed death in yeast, Fabrizio et al. (2004) advance this idea by linking the ability to die with the ability to genetically adapt to the adverse environment. They found that if a large portion of the population in a culture of starving yeast has the ability to die early (before they would die from the lack of nutrients), then the culture is more likely to eventually regrow and survive long term. For this to occur, the dying cells presumably activate a programmed death pathway triggered by mitochondrial superoxide, and their "debris" nourishes the rare surviving genetic variant (Figure 1B). If true, the ability of yeast to undergo programmed cell death would confer an evolutionary advantage for the species. Herker et al. (2004) argue that this process requires the yeast metacaspase/Yca1.

One could argue that this rare variant only survives because it is a "cheater," a self-preservationist that has simply lost the ability to die and is not otherwise better adapted for its new environment. However, death-resistant yeast, such as the deletion mutants *yca1*Δ, *ras2*Δ, and *sch9*Δ, and yeast overexpressing SOD1 (superoxide dismutase) or mammalian Bcl-2 (that only start dying after 5–10 days in depleted medium), have little or no ability to regrow, despite their improved short-term survival (Fabrizio et al., 2004; Herker et al., 2004). That is, delayed death of yeast cells in these cultures apparently does not allow for the adaptation/selection process, resulting in slow death of the entire culture within 40–60 days. These findings offer potential explanations for why extended lifespan is not selected for during evolution, and why programmed cell death may be an ancient process, preceding multicellular organisms.

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Atonal Points the Way—Protein-Protein Interactions and Developmental Biology

Many cells maintain their state of determination long after the signals that induced it decay. In this issue of *Developmental Cell*, zur Lage and colleagues describe how certain cells sustain proneural gene expression through direct interactions between transcription factors.

Cell fate determination is so often discussed from the perspective of extracellular inducing signals and the responses cells make to them that one could be forgiven

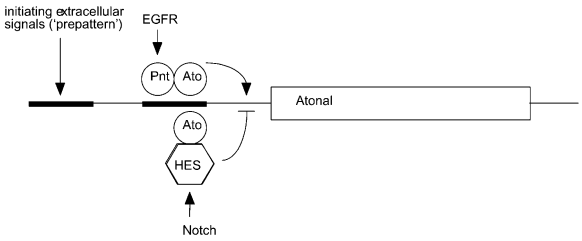

Figure 1. Minimal Scheme for Control of Proneural Gene Autoregulation by Protein-Protein Interactions on the Enhancer

for thinking that the extracellular signals and their interpretation constituted the whole mechanism. It is worth remembering that, by definition, cell fate determination occurs when fate becomes *independent* of the available extracellular environments (Slack, 1983). Maintenance of cell fates after the inducing signals dissipate is a distinguishing feature of multicellular differentiation. Some form of self-sustained gene expression is required to maintain expression of regulatory genes that define cell fate and underlies the widespread importance of autoregulatory gene expression in cell fate determination. Regulatory gene expression that is not autoregulatory is just prepattern (indeed, this provides as good a definition of the "prepattern" concept as any). Sustained gene expression can be achieved by binding of a transcription factor to its own promoter, although other cell-autonomous mechanisms are also possible.

Now, zur Lage and colleagues have described in molecular detail how particular neural cells become independent of extracellular signals in the fruit fly *Drosophila melanogaster* (zur Lage et al., 2004 [this issue of *Developmental Cell*]). The subject of their analysis is a proneural gene, *atonal*, which encodes a bHLH transcription factor. Atonal is a convenient choice for study because it is necessary and sufficient for the determination of several classes of neural precursor cells in *Drosophila*, unlike other similar proneural genes that act redundantly so that their expression is maintained by crossregulatory interactions that are more difficult to decipher. Atonal functions in neural precursor determination within several different sensory organs, including the chordotonal stretch receptors that are the focus of this study.

Transcription of the *atonal* gene depends on multiple enhancers (Sun et al., 1998). These divide into two groups (see Figure 1): some are active in the absence of functional *atonal* product and are involved in establishing *atonal* expression in response to extracellular signals in particular proneural regions; others depend on *atonal* product, and so mediate the "autoregulation" that maintains *atonal* expression independent of extracellular signals. A seminal finding was that multiple separate *atonal*-dependent enhancers mediated the autoregulation of *atonal* in distinct tissues (Sun et al., 1998). The implication was that no autoregulatory enhancer could be activated by Atonal alone, because any enhancer that was so simple would be active wherever Atonal was expressed. Rather, each enhancer must identify a distinct route to maintaining expression that involves other factors besides Atonal.

zur Lage et al. have now analyzed one enhancer in detail and shown that it is active whenever Atonal is expressed in a cell where the EGF receptor pathway is also active. The 367 bp enhancer activity depends on one binding site for Ato and one for the ETS domain protein Pointed (Pointed is a common transcriptional target of EGF receptor signal transduction). Remarkably, the relevant Ato and Pnt binding sites are only 4 bp apart, and this proximity is conserved in *atonal* regulatory sequences from other Drosophilid species. The tandem Ato/Pnt binding sites alone were able to reconstitute much of the enhancer function of the 367 bp sequence. Gel mobility shift assays confirmed the existence of a ternary Ato/Pnt/DNA complex and supported synergistic DNA binding by the two transcription factors. By modeling Ato and Pnt on the known structures of other bHLH and ETS domain proteins, zur Lage et al. identify a putative interaction surface between Ato and Pnt that may be specific for the Ato class of bHLH proteins.

Cooperative binding of transcription factors is nothing new. Indeed, there is a prior example of interaction between bHLH and Ets protein in HIV transcription (Sieweke et al., 1998). The significance lies in the mechanism of fate determination that emerges. zur Lage et al. demonstrate that a local EGFR signal acts combinatorially with more widespread Ato prepattern to raise particular cells over the threshold for Ato autoregulation. Such cells are enabled to maintain neural fate, while in other nearby cells *atonal* transcription remains dependent on other enhancers and is lost as the transient inputs to these enhancers fade. It is not yet clear whether the EGFR requirement is needed only transiently to push *atonal* expression over a threshold. Alternatively, EGFR may be needed continuously until neural differentiation has become *atonal* independent, raising the question of how Pointed activity itself is maintained. In either case, the mechanism places some molecular flesh on the bare-boned concept of cell fate determination.

One obvious question is how many of the other myriad roles of the EGFR pathway in fate determination act similarly. If only sequence analyses alone were sufficient to predict other similar regulatory elements! Unfortunately, consensus sequences for Pnt or Ato binding are short and abundant, and even the 367 bp studied here contain other such sequences that were found not to be functionally important. Other DNA sequences have been found in autoregulatory DNA from other proneural genes, suggesting analogous combinatorial inputs, but the proteins that act through these sequences have yet to be identified (Culi and Modolell, 1998).

In addition to responding to cooperative signals, there is a second reason why autoregulation provides a nodal point for regulation (Figure 1). At the same time as conferring independence from initiating signals, autoregulation heightens sensitivity to inhibitory signals, because blocking autoregulation is an efficient and potentially irreversible mechanism of extinguishing gene expression. This may be how the Notch pathway blocks neural fate determination. Because some HES proteins (transcriptional repressors induced by Notch signaling) interact directly with proneural proteins, they can be brought to the DNA via autoregulatory enhancers (Giagtzoglou et al., 2003). Notch signaling is therefore most effective

at blocking transcription of proneural genes when transcription becomes autoregulatory, interrupting fate determination at this crucial juncture (Baker et al., 1996; Culi and Modolell, 1998). This is a further example where the protein-protein interactions between transcription factors determine the biology. As zur Lage et al. point out, it may prove important whether any Ato/HES interactions exclude interactions such as that between Ato and Pointed. Evidently, further insights into the mechanisms of cell fate determination lie ahead.

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Bub1, a Gatekeeper for Cdc20-Dependent Mitotic Exit

The mitotic spindle assembly checkpoint arrests cells at metaphase by suppressing Cdc20, a protein required to trigger ubiquitination and consequent degradation of cyclin B. New evidence from Tang et al. appearing in the November 5th issue of *Molecular Cell* finds that one of the checkpoint proteins, Bub1, specifically phosphorylates Cdc20 to suppress APC/C activation.

The cell cycle is subject to a number of checkpoint controls that function to preserve the genome by restraining progression until prerequisite events have been properly completed. From yeast to mammals there are spindle assembly checkpoints that read proper alignment and tension of chromosomes in the mitotic spindle before anaphase can initiate. The system appears to function by the recruitment of a group of checkpoint control proteins, including (in higher eukaryotes) Mad1, Mad2, Bub1, Bub3, Mps1, and BubR1, to the kinetochores, and ablation or suppression of function of any of these proteins substantially compromises mitotic checkpoint control (Lew and Burke, 2003). Ultimately, the checkpoint operates by sequestering Cdc20, a key regulator of the anaphase promoting complex/cyclosome (APC/C), a complex that functions to ubiquitinate two key substrates, securin and cyclin B, tagging them for proteosome destruction, that in turn is the critical event permitting mitotic exit (Peters, 2002).

Three of the checkpoint control proteins, Bub1, Mps1, and BubR1, are protein kinases. It has been reasonable to assume that kinase activity is intimately connected to checkpoint function, but the crucial substrates these checkpoint proteins regulate, that make sense with respect to checkpoint control, have been lacking. Indeed, the kinase domain of BubR1 appears to be dispensable for its APC/C inhibitory activity (Tang et al., 2001). Given the number of checkpoint control proteins involved, it would appear that the control network, once unraveled, would turn out to be highly complex. Cdc20 phosphorylation has recently received attention for its potential in regulating APC/C activation (Chung and Chen, 2003), but the protein kinase identified, MAPK, is not one of the checkpoint kinases.

Contrary to the expected complexity of checkpoint control, a striking and important paper by Tang et al., appearing in the November 5th issue of *Molecular Cell*, has shed unexpected light on the mechanism by making a clear linkage between the kinase activity of one of the checkpoint control proteins, Bub1, and Cdc20 control of APC/C activation. In this paper, the authors have established that, for mammalian cells, there are six phosphorylation sites on Cdc20 that are phosphorylated by Bub1, but not by BubR1, MAPK, or a battery of other kinases. Further, mutation of these phosphorylation sites to alanine creates a dominant-negative effect, with reduced checkpoint arrest in mitosis. Going in the other direction, the authors have established that Bub1 ablation, or expression of a Bub1 dead kinase, abolishes Cdc20 phosphorylation and also suppresses the spindle assembly checkpoint.

As the authors point out, the spindle assembly checkpoint is exquisitely sensitive, responding to a single off-plate chromosome or to loss of tension in properly aligned chromosomes. The existence of such catalytic checkpoint machinery, as described here, offers a highly sensitive response mechanism that should permit the necessary rapid amplification of signal.

While this work establishes a clear pathway by which the spindle assembly checkpoint may at least partly control APC/C function, it opens many important questions. Issues of great interest include how this pathway fits with the essential functions of the other checkpoint control proteins and of Cdc20 phosphorylation by other protein kinases (Chung and Chen, 2003). Further, it will be important to address what controls Bub1 so that it maintains Cdc20 in phosphorylated status only during checkpoint arrest. Bub1 and Cdc20 participate in a multiprotein complex composed of other checkpoint